Auranofin Inhibition of Thioredoxin Reductase in a Preclinical Model of Small Cell Lung Cancer

Spenser S. Johnson, Dijie Liu, Jordan T. Ewald, Claudia Robles-Planells, Khaliunaa Bayanbold, Brian R. Wels, Shane R. Solst, M. Sue O'Dorisio, Bryan G. Allen, Yusuf Menda, Douglas R. Spitz, Melissa A. Fath

Running Head: Auranofin Inhibition of TrxR in SCLC Xenografts

Abstract

Thioredoxin Reductase (TrxR) is a key enzyme in reactive oxygen species (ROS) detoxification and in redox regulation. Because cancer cells produce increased steady-state levels of ROS (i.e., superoxide and hydrogen peroxide), TrxR is viable target in clinical trials using the anti-rheumatic drug, auranofin (AF). To extend these observations to small cell lung cancer (SCLC), AF-mediated TrxR inhibition as well as tolerability and tumor growth inhibition was determined in a xenograft model. AF was administered intraperitoneal, daily or twice daily for 1 to 5 days in mice bearing DMS273 xenografts. AF uptake was determined by mass spectrometry of gold and inhibition of TrxR in the tumor was determined. The optimal dose was 4 mg/kg once daily resulting in 18 μM gold in the plasma and 50% inhibition of TrxR activity in DMS273 SCLC tumors. This regimen given for 14 days provided a trend for prolonged median survival from 17.5 to 22 days ($p=0.058$, N=20 controls, 19 AF) without causing changes in bodyweight, bone marrow toxicity, blood urea nitrogen or creatinine. These results support the hypothesis that AF is an effective inhibitor of TrxR and suggest that AF could be used as an adjuvant in radio-chemotherapy protocols to enhance therapeutic efficacy.

Introduction

Lung cancer is the second most common cancer and small cell lung cancer (SCLC) makes up about 15% of lung cancer diagnoses. SCLC is a high-grade neuroendocrine tumor with characteristics of rapid tumor growth

43 and high vascularity; two-thirds of patients have distant metastatic disease at initial diagnosis. Standard of care
44 therapies for limited stage disease include cisplatin or carboplatin in combination with etoposide and thoracic
45 irradiation. Improved outcomes with immunotherapy as concurrent primary or adjuvant therapy has been
46 demonstrated but these combination therapies are still considered experimental.[1 2] The median survival duration
47 remains less than two years for patients with limited-stage disease and less than one year for patients with
48 metastatic disease.[3] Therefore, improved treatment options are of utmost importance for SCLC.

49 A hallmark of cancer is the increased steady-state levels of reactive oxygen species (ROS) often stemming
50 from one electron reductions of O_2 in mitochondrial electron transport chains to form superoxide radical ($O_2^{\cdot -}$)
51 which is rapidly converted to H_2O_2[4]. Cancer cells adapt to increased levels of ROS by upregulating endogenous
52 antioxidant systems, including the scavenging enzymes superoxide dismutase and catalase, as well as enzymes
53 in the glutathione-dependent and thioredoxin-dependent hydroperoxide metabolic pathways. The glutathione
54 and thioredoxin systems play key roles in the overall cellular oxidation state because of their intracellular dithiol
55 recycling nature.[5] In particular, the thioredoxin system consists of the redox-active proteins thioredoxin (Trx)
56 and the NADPH electron acceptor thioredoxin reductase (TrxR) which primarily function to reduce
57 peroxiredoxin (Prx) during the catalytic reduction of hydroperoxides. TrxR has three known mammalian
58 isoforms that can be found in the cytosol mitochondria, and one specific to spermatozoa.[6] According to the
59 Project Score database, knock-out of TrxR has shown an anti-tumoral effect in 21% of 324 cell-lines subjected
60 to CRISPR-Cas9 screens [7] clearly indicating TrxR as a potential target for cancer treatment.

61 Originally developed in the 1980s for rheumatoid arthritis, auranofin (AF) was thought to exert it effects by
62 inhibiting humoral immunity.[8] The most common adverse reactions associated with AF treatment were
63 gastrointestinal-as loose stools or diarrhea occurred in 39% of patients. Rashes and pruritis were also common,
64 occurring in >10% of patients.[8 9,10] In addition, gold salts caused thrombocytopenia in 0.7% of patients.[11]

65 Although diarrhea and stomach upset occurred in the initial month of treatment, other adverse events typically
66 occurred only after prolonged therapy.[12] Clinical AF use has declined due to the availability of more effective
67 and better tolerated biologic treatments for rheumatoid arthritis.

68 AF, a gold phosphine compound, was recently discovered to irreversibly inhibit both cytosolic and
69 mitochondrial TrxR.[13,14] This discovery has led to the active investigation of repurposing AF as an anticancer
70 agent. Currently, four clinical trials using AF are underway for chronic lymphocytic leukemia (NCT01419691,
71 NCT01747798), ovarian cancer (NCT03456700), and recurrent lung cancer (NCT01737502). We have
72 previously shown that treatment of breast cancer cells with AF before external beam radiation decreases the
73 number of invading metastatic breast cancer cells as well as the number of breast cancer stem cells.[15] We also
74 demonstrated the suppressing TrxR with AF can sensitize breast cancer stem cells to ROS induced
75 differentiation and cytotoxicity.[16]

76 The current study developed a SCLC xenograft model in female athymic nude mice and determined a safe
77 and efficacious dose of AF that significantly inhibited TrxR in tumors. Complete blood counts, bone marrow
78 hematopoietic stem cell and progenitor cell populations, renal and liver function evaluation by serum chemistry
79 analyses, weight loss, and animal body conditioning as well as survival were examined after two-week dosing
80 schedule of AF. These results provide a rigorous characterization of this SCLC xenograft model for testing the
81 FDA approved thioredoxin reductase inhibitor, AF, as an adjuvant to radiation therapy in a preclinical setting.
82

83 **Methods and Materials**

84 Cell Culture

85 DMS273 human small cell lung cancer cells were obtained from American Type Culture Collection
86 (ATCC) and maintained in RPMI 1640 media (Mediatech Inc., Manassas, VA) with 10% fetal bovine serum

87 (Atlanta Biologicals, Lawrenceville, GA). Cultures were maintained in 5% CO_2 and humidified in a 37°C
88 incubator. Cells were used within 20 passages from ATCC and tested as mycoplasma negative.

89 Animal Experiments

90 Female 6–8-week-old athymic-nu/nu nude mice were purchased from Envigo. Mice were housed in a
91 pathogen-free barrier room in the Animal Care Facility at the University of Iowa and handled using aseptic
92 procedures. All procedures were approved by the IACUC committee of the University of Iowa and conformed
93 to the guidelines established by NIH. Mice were allowed at least one week to acclimate prior to beginning
94 experimentation; food and water were made freely available. Tumor cells were inoculated into nude mice by
95 subcutaneous injection of 0.1 mL aliquots of saline containing 1×10^6 DMS273 cells into the right and/or left
96 flank using 25-gauge needles. When tumor volumes measured approximately 100 mm^3 treatments were started.
97 Mouse health was monitored daily during drug treatment. Mice were terminally anesthetized using 200 µl
98 intraperitoneal injection of ketamine/xylazine 17.5/2.5 µg/µl mix. When analgesia was achieved, as determined
99 by lack of toe pinch response, blood was withdrawn via cardiac puncture followed by cervical dislocation.

100 In vivo AF Dose Escalation

101 In the dose escalation study, five groups of three mice/group were given AF intraperitoneal (IP) once
102 (QD) or twice (BID) a day for 5 days. AF was made by dissolving 5.0 mg AF in 250 µl of absolute ethanol in a
103 10 ml glass beaker. When completely dissolved 250 µl Kolliphor EL (Sigma C5135) and 4 ml of 0.9% normal
104 saline for injection (Hospira) were added and pH was adjusted to 7-8 range using approximately 25 µl 8.4%
105 sodium bicarbonate for injection (Hospira) followed by addition of normal saline for injection to adjust final
106 volume to 8.33 ml and filtered using 200-micron filter into a sterile vial. The final AF concentration was 0.6
107 mg/ml and it was made fresh daily. Mice in the control group were administered vehicle without drug. Mice
108 were euthanized 21 hours after the last QD dose and 9 hours after the last BID dose; tumors and organs were

109 frozen in liquid nitrogen for later evaluation for TrxR activity. Terminal blood was collected via cardiac
110 puncture under anesthesia and plasma separated via centrifugation at 10,000 rcf for 10 min.

111 Biochemical Assays

112 Glutathione peroxidase 1 (GPx1) activity was determined spectrophotometrically using the method of
113 Lawrence and Burk.[15] Enzymatic activity was determined by measuring the disappearance of NADPH at 340
114 nm in the presence of GPx1 from cell pellets or standards. One unit of activity was defined as 1 μmole NAPDH
115 oxidized/min at specified glutathione (GSH) concentration. Protein concentrations were determined by the
116 Lowry assay.

117 TrxR activity was determined spectrophotometrically using the reduction of 5,5'-dithiobis(2-
118 nitrobenzoic) acid (DTNB) with NADPH to 5-thio-2-nitrobenzoic acid (TNB), which generates a yellow color
119 at 412 nm. (Millipore-Sigma CS0170). Enzymatic activity was determined by subtracting the time dependent
120 increase in absorbance at 412 nm in the presence of the TrxR activity inhibitor, aurothioglucose from total
121 activity. One unit of activity was defined as 1 μM TNB formed/(min·mg protein). Protein concentrations were
122 determined by the Lowry assay.

123 Determining Gold content in Plasma

124 Plasma samples were prepared by treatment with aqua regia followed by dilution and analysis using
125 inductively coupled plasma mass spectrometry (ICPMS). Aqua regia was prepared from Optima grade (Fisher
126 Scientific) hydrochloric and nitric acids (3:1, respectively). A 100-μL aliquot of plasma was transferred to a
127 15-mL centrifuge tube, 500 μL aqua regia added and the mixture heated on a hot block at 90 +/- 4 °C for 30
128 minutes. After cooling, volume was brought to 2.0 mL using deionized water. A 500-μL aliquot of the
129 digestate was mixed with an equal volume of internal standard (10 μg/L Iridium) and analyzed using ICPMS
130 (Agilent 7700) with an external calibration curve.

131 Tumor growth and survival

132 In the survival experiments, 2 groups of mice (9-10/group) were administered AF at 4 mg/kg or vehicle
133 interperitoneally once a day for 14 consecutive days. Mice were monitored and tumors measured daily using
134 Vernier calipers (Vol = (Length x Width^2)/2) and euthanized on the day when tumor size exceeded 1000 mm^3.
135 No mice died before reaching this endpoint. This experiment was repeated two times. Three mice in each group
136 were euthanized 24 hours after the last dose of AF, and tumors were frozen at -80 ºC for TrxR activity assay.
137 Blood was collected via cardiac puncture under, see above, and plasma separated via centrifugation at 2500 rcf
138 for 10 min and sent to Antech Diagnostics for clinical chemistry evaluation. Additional blood was obtained via
139 mandibular vein and diluted 1:10 in PBS and a complete blood cell count (CBC) processed in a Siemens
140 ADVIA 120 Hematology Analyzer.

141 Bone marrow harvest and flow cytometry

142 Immediately after death, whole bone marrow cells were obtained by flushing one femur with PBS 2%
143 FBS followed by erythrocyte elimination with ACK Lysing Buffer (10-548E, BioWhittaker, Lonza). The
144 remaining cells were filtered through 40 µm pore size cell strainer and counted. 1x10^6 cells were stained with
145 Zombie Aqua (423102, BioLegend) for 20 min at room temperature for live/dead cells discrimination, followed
146 by a 30 min surface staining on ice using PBS 1% BSA as staining buffer with allophycocyanin (APC)-
147 conjugated, lineage negative (Lin-) cocktail (BD Bioscience), PerCP/Cy5.5-conjugated anti-Sca1 (Clone D7,
148 BioLegend), APC/Cy7-conjugated anti-cKit (Clone 2B8, BioLegend), PE-conjugated anti-CD135 (Clone
149 A2F10, BioLegend), FITC-conjugated anti-CD48 (Clone HM48-1, BioLegend), PE/Cy7-conjugated anti-
150 CD150 (Clone TC15-12F12.2, BioLegend) and BV421-conjugated anti-CXCR4 (Clone L276F12, BioLegend).
151 Cells were immediately analyzed using an LSR II flow cytometer (Becton Dickinson). The biomarkers were
152 applied to identify multipotent hematopoietic stem cells (HSC) (Lin- cKit+ Sca1+, LSK), ST-HSC (short-term

153 hematopoietic stem cells, Lin- cKit+ Sca1+ CD135- CD48- CD150-), LT-HSC (long-term hematopoietic stem
154 cells, Lin- cKit+ Sca1+ CD135- CD48+CD150-), and committed progenitors (hematopoietic stems and
155 progenitor cells, HSPC, Lin- cKit+ Sca1+ CD135-) using FlowJo Software (Treestar, Ashland, OR).

156 Statistical analysis

157 The biochemical data analysis was performed using ANOVA multiple comparisons with uncorrected
158 Fishers LSD on Graph Pad Prism software. The *in vivo* analysis regression analysis was used to model tumor
159 growth as a nonlinear function of follow-up time and to make treatment group comparisons of estimated tumor
160 means. Survival graphs were obtained with the methods of Kaplan–Meier and compared with log-rank tests.

161

162 **Results**

163 Dosing AF to a Maximal Effective Concentration in mice

164 Initially, we sought to determine a safe and effective dose of AF in mice with SCLC cell line DMS273
165 xenografts. AF (2 or 4 mg/kg) was administered IP once (QD) or twice (BID) daily up to 5 days. Plasma gold
166 levels were measured nine hours after the last BID dosing or 21 hours after the last QD dosing. There was a
167 significant increase in plasma gold content of all mice treated with AF **(Figure 1)**. As expected, 4 mg/kg dosing
168 resulted in significantly more gold in the plasma than 2 mg/kg dosing. There was not a significant difference
169 between QD and BID dosing indicating that steady state levels of gold in the plasma can be maintained with
170 five days of QD dosing as expected with the long half-life of AF reported in humans and rodents **(Figure 1)**.[8,17]
171 To investigate if these plasma levels are effective at reducing TrxR activity we measured the activity in the
172 DMS273 tumors. After 24 hours, AF had no effects on TrxR activity in the tumors, but by 5 consecutive days of
173 dosing TrxR activity was inhibited by 50% at 2 mg/kg BID, 4 mg/kg QD, and 4 mg/kg BID **(Figure 2A)**. Gpx1
174 is also a selenium-containing enzyme with an active thiol moiety, and it has been reported that high doses of AF

175 can inhibit its activity.[18] As a control for AF specificity for inhibition of TrxR, GPx1 activity was measured in
176 the tumors after five days in the 4 mg/kg groups; there was no change in GPx1 activity **(Figure 2B)**. TrxR
177 activity in kidneys was significantly reduced by 2 mg/kg QD and 4 mg/kg BID at 24 hours. TrxR activity in
178 kidneys was significantly inhibited by 5 consecutive days of dosing by 50% in 2 mg/kg BID, 4 mg/kg QD, and
179 4 mg/kg BID **(Figure 3A)**. TrxR inhibition in liver was only observed at an AF dose of 4 mg/kg BID at five
180 days **(Figure 3B)**. No significant changes in weight or general health in any mice were observed (data not
181 shown). Together these results suggest that 4 mg/kg QD is the optimal AF dose to achieve inhibition of TrxR
182 activity in DMS273 tumors. Importantly, this dose also inhibited TrxR activity in the kidneys indicating
183 potential nephrotoxicity.

184 AF Effects on Tumor Growth, Survival, and Normal Tissue Injury

185 DMS273 cells were implanted subcutaneously into the right flank of nude female mice and allowed to
186 grow for 6 days followed by treatment with AF at 4 mg/kg or vehicle control for 14 consecutive days. Tumor
187 volumes and mice weights were measured every other day or daily as the tumor size progressed. Twenty-four
188 hours after the last AF dose, 4 mice were euthanized, and their tumors collected to determine TrxR activity. AF
189 significantly inhibited TrxR activity in the tumors by 75%, and this inhibition was independent of tumor volume
190 **(Figure 4A & B)** indicating excellent penetration of the drug even in large tumors. When the tumors reached
191 1000 mm^3 the mice were euthanized. The experiment was repeated in a second cohort of mice in an identical
192 fashion. Kaplan Meier survival curves combining both cohorts resulted in an encouraging trend toward
193 increased survival (median overall survival of 18 days in control versus 22 days in drug treated animals;
194 p=0.058) (**Figure 5A**). Importantly, 14 days of continuous AF treatment did not result in changes in weight,
195 body conditioning or general activity level in any mouse (data not shown). CBC data on day 14 following AF
196 therapy (**Table 1**) demonstrated no significant toxicity of the drug. At euthanasia, bone marrow sub-population

197 analyses demonstrated no change of HSCs or HSPCs portions in the AF treatment group as compared to the
198 control group ($P > 0.05$) (**Figures 5B)**, and no significant change of LT-HSC or ST-HSC population (Data not
199 shown), suggesting the bone marrow sustained the stem cell pool by self-renewal and the potential to
200 differentiate to multipotent progenitors as well as HSPCs. Combining with the unchanged effector cells
201 population by CBC data, it demonstrated that AF treatment didn't cause hematotoxicity. Lastly, at euthanasia
202 common markers of normal tissue injury damage for both the kidney (total serum protein, blood urea nitrogen
203 and creatine) and liver (alkaline phosphatase, alanine transaminase and bilirubin) in the plasma of mice,
204 demonstrated no significant differences with AF treatment **(Figure 5C).** These results suggest that this dosing
205 regimen is nontoxic to kidneys, liver, and bone marrow as well as demonstrating a trend toward a survival
206 advantage in tumor bearing animals.
207

208 **Conclusion**

209 In non-malignant cells, redox metabolism is tightly coupled via non-equilibrium steady-state fluxes of
210 reactive metabolic by-products and electron carriers through redox sensitive signal transduction pathways. It is
211 increasingly recognized that dysregulation of mitochondrial redox metabolism in cancer cells leads to the
212 increased steady-state levels of reactive species including H_2O_2 and organic hydroperoxides.[19] Ionizing
213 radiation leads to the formation of free radicals from the radiolysis of H_2O to form hydroxyl radical. We are
214 investigating repurposing FDA approved redox active drugs that selectively disrupt the oxidative balance in
215 cancer cells as potential radio-chemo-sensitizers to take advantage of the known dysregulation of oxidative
216 metabolism in cancer cells to improve therapy outcomes.[15,20,21] In this paper the efficacy of AF treatment in a
217 preclinical xenograft mouse model of SCLC was characterized for future studies as a radio-chemo-sensitizer to
218 improve outcomes in this deadly disease.

219 These studies demonstrated that AF treatment resulted in dose dependent increases in plasma drug
220 concentration that led to significant inhibition in TrxR in DMS273 xenograft tumors after five days. The
221 average level of the gold found in the plasma with 4 mg/kg IP dosing, 18.4 μM in mice is eleven-fold the
222 average concentration found in humans taking the recommended dose of 6 mg/day orally for seven days, 1.6
223 μM.[22] The long half-life (35 days) of AF suggests that higher serum and tumor drug levels can be safely
224 achieved with higher daily dosing and/or continued dosing.[22] In fact, the primary objective of an ongoing
225 Clinical trial (NCT01737502) is to establish the maximum tolerated dose of AF in combination with sirolimus
226 in lung cancer, including SCLC, with an estimated completion in August, 2023.
227 AF dosing of 14 days of 4 mg/kg daily IP in female nude mice demonstrated a highly significant 75%
228 reduction in TrxR that was independent of tumor size indicating the AF could penetrate the tumor efficiently.
229 This dose was also well tolerated as demonstrated by general health of the mice, no weight loss, no effect on
230 bone marrow progenitor cells or changes in CBC. In addition, plasma chemistry indicated that there was no
231 evidence of damage to the kidneys or liver. The 14-day AF dosing schedule did demonstrate a trend toward an
232 increase in median overall survival that did not reach statistical significance (p=0.058). Overall, since thoracic
233 irradiation combined with chemotherapy continues to be a standard of care for limited stage SCLC, and is
234 known to induced metabolic oxidative stress in cancer cells, the current data continues to support the hypothesis
235 that AF-induced inhibition of TrxR could represent a promising redox active adjuvant for selective tumor radio-
236 chemo-sensitization.
237
238 **Figure Legends**
239 **Figure 1 - Gold circulates through blood plasma after IP injections.** Mice were injected IP with AF for five
240 days. Plasma samples were collected 9 hours after final BID dose and 21 hours after final QD dose. Gold was

241 determined using inductively coupled plasma mass spectrometry ** = p-value < 0.01, **** = p-value < 0.0001.
242 Analyzed by One-Way ANOVA multiple comparison.

243 **Figure 2 – Auranofin selectively decreases TrxR activity in SCLC xenografts.** Female athymic nude mice
244 were xenografted with 5 x10^6 DMS273 cells and given AF IP injections after 5 days either QD or BID. Kidney,
245 liver, and tumor samples collected for TrxR activity; results are plotted after 24 hours or 5 days of treatment. *
246 = p-value < 0.05, ** = p-value < 0.01, *** = p-value < 0.001. Analyzed by Mix Effects ANOVA with Fishers
247 LSD comparisons.

248 **Figure 3 – 4mg/kg QD doses of AF results in a decrease in TrxR activity in mouse kidneys but not liver.**
249 Female athymic nude mice were xenografted with 5 x10^6 DMS273 cells and given AF IP injections for 5 days
250 either once daily (QD) or twice daily (BID). Kidney, liver, and tumor samples were collected at euthanasia and
251 evaluated for TrxR activity; results are plotted after 24 hours or 5 days of treatment. * = p-value < 0.05, ** = p-
252 value < 0.01, *** = p-value < 0.001. Analyzed by two-way ANOVA with Fishers LSD comparisons.

253 **Figure 4 – AF decreases TrxR activity in DMS273 tumors in vivo independent of tumor size.** Small cell
254 lung cancer (DMS273) xenografts were treated with Aur 4 mg/kg QD for 12 days then euthanized and
255 evaluated for TrxR enzyme activity assays and results are plotted in aggregate (A) or as a function of tumor size
256 at the end of treatment (B). * p<0.05 compared to untreated cells two-ways ANOVA with Fishers LSD
257 comparisons.

258 **Figure 5 – AF increases survival in DMS273 xenograft mice without causing toxcity.** Female nude mice
259 bearing DMS273 xenografts were treated with either vehicle or 4 mg/kg AF I.P every day for 14 days. Mice
260 were euthanized when tumors were ≥ 1000 mm^3. **A)** Kaplan-Meier curves were used to estimate survival. Log-
261 rank (Mantel Cox test) for significance. **B)** Bone marrow was harvested from the femur at euthanasia and

262 subjected to flow cytometry for HSCs (Lineage (Lin)− Sca-1+ c-Kit+, LSK) and HSPCs (Lin- cKit+ Sca1+

263 CD135-) populations. **C)** blood was draw via cardiac puncture at euthanasia and evaluate for clinical chemistry.

264

265 **Acknowledgments**

266 We would like to thank the Holden Comprehensive Cancer Center; the University of Iowa College of Medicine

267 Flow Cytometry Facility; the University of Iowa Office of Animal Resource; the University of Iowa Free

268 Radical Enzyme Core; the Holden Comprehensive Cancer Center Biostatistical Core.

269 **Funding**

270 The authors disclosed receipt of the following financial support for the research, authorship, and/or publication

271 of this article: NCI: P01 CA217797, R50 CA243693

272 SPORE: P50-CA174521, P30 CA086862 and P50 CA174521-05S2

273 **References**

274

275

276 1. Lu M, Zhang R, Qi LS, Wang YL, Sun XX, You J. Pathologic responses to neoadjuvant
277 chemoimmunotherapy in primary limited-stage small-cell lung cancer. *Thorac Cancer*. Nov 2022;13(22):3208-
278 3216. doi:10.1111/1759-7714.14679
279 2. Yao N, Qin Z, Ma J, et al. Anti-programmed death ligand 1 immunotherapy in patients with limited-
280 stage small cell lung cancer: a real-world exploratory study. *J Chemother*. Sep 20 2022:1-7.
281 doi:10.1080/1120009X.2022.2125750
282 3. Rudin CM, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. *Nat Rev Dis Primers*. Jan 14
283 2021;7(1):3. doi:10.1038/s41572-020-00235-0
284 4. Zhou D, Shao L, Spitz DR. Reactive oxygen species in normal and tumor stem cells. *Advances in cancer*
285 *research*. 2014;122:1-67. doi:10.1016/B978-0-12-420117-0.00001-3
286 5. Holmgren A, Johansson C, Berndt C, Lonn ME, Hudemann C, Lillig CH. Thiol redox control via
287 thioredoxin and glutaredoxin systems. *Biochem Soc Trans*. Dec 2005;33(Pt 6):1375-7.
288 6. Lu J, Holmgren A. The thioredoxin antioxidant system. *Free radical biology & medicine*. Jan
289 2014;66:75-87. doi:10.1016/j.freeradbiomed.2013.07.036

7. Behan FM, Iorio F, Picco G, et al. Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. *Nature*. Apr 2019;568(7753):511-516. doi:10.1038/s41586-019-1103-9
8. Furst DE. Mechanism of action, pharmacology, clinical efficacy and side effects of auranofin. An orally administered organic gold compound for the treatment of rheumatoid arthritis. *Pharmacotherapy*. Sep-Oct 1983;3(5):284-98. doi:10.1002/j.1875-9114.1983.tb03277.x
9. Horton RJ. Comparative safety and efficacy of auranofin and parenteral gold compounds: a review. *Scandinavian journal of rheumatology Supplement*. 1983;51:100-10. doi:10.3109/03009748309095361
10. Liu J, Akahoshi T, Namai R, Matsui T, Kondo H. Effect of auranofin, an antirheumatic drug, on neutrophil apoptosis. *Inflamm Res*. Sep 2000;49(9):445-51. doi:10.1007/s000110050615
11. Bakke E, Myklebust G, Gran JT. [Thrombocytopenia induced by auranofin treatment]. *Tidsskr Nor Laegeforen*. Nov 20 1997;117(28):4081-2. Trombocytopeni utlost ved auranofinbehandling.
12. Bandilla KK, Missler B. Longterm results of auranofin therapy. *Clin Rheumatol*. Sep 1987;6 Suppl 2:35-42. doi:10.1007/BF02203383
13. Rigobello MP, Scutari G, Folda A, Bindoli A. Mitochondrial thioredoxin reductase inhibition by gold(I) compounds and concurrent stimulation of permeability transition and release of cytochrome c. *Biochemical pharmacology*. Feb 15 2004;67(4):689-96.
14. Gromer S, Arscott LD, Williams CH, Jr., Schirmer RH, Becker K. Human placenta thioredoxin reductase. Isolation of the selenoenzyme, steady state kinetics, and inhibition by therapeutic gold compounds. *The Journal of biological chemistry*. Aug 7 1998;273(32):20096-101. doi:10.1074/jbc.273.32.20096
15. Rodman SN, Spence JM, Ronnfeldt TJ, et al. Enhancement of Radiation Response in Breast Cancer Stem Cells by Inhibition of Thioredoxin- and Glutathione-Dependent Metabolism. *Radiation research*. Oct 2016;186(4):385-395. doi:10.1667/RR14463.1
16. Luo M, Shang L, Brooks MD, et al. Targeting Breast Cancer Stem Cell State Equilibrium through Modulation of Redox Signaling. *Cell Metab*. Jul 3 2018;28(1):69-86 e6. doi:10.1016/j.cmet.2018.06.006
17. Van Riel PL, Gribnau FW, Van de Putte LB, Arts CW, Van Aernsbergen A. Serum gold concentrations during treatment with auranofin. Research Support, Non-U.S. Gov't. *Clinical rheumatology*. Mar 1987;6(1):50-4.
18. Chaudiere J, Wilhelmsen EC, Tappel AL. Mechanism of selenium-glutathione peroxidase and its inhibition by mercaptocarboxylic acids and other mercaptans. Research Support, U.S. Gov't, P.H.S. *The Journal of biological chemistry*. Jan 25 1984;259(2):1043-50.
19. Spitz DR. Manipulations of Redox Metabolism for Enhancing Radiation Therapy Responses: A Historical Perspective and Novel Hypothesis. *Semin Radiat Oncol*. Jan 2019;29(1):1-5. doi:10.1016/j.semradonc.2018.10.010
20. Sciegienka SJ, Solst SR, Falls KC, et al. D-penicillamine combined with inhibitors of hydroperoxide metabolism enhances lung and breast cancer cell responses to radiation and carboplatin via H2O2-mediated oxidative stress. *Free radical biology & medicine*. Jul 2017;108:354-361. doi:10.1016/j.freeradbiomed.2017.04.001
21. Falls-Hubert KC, Butler AL, Gui K, et al. Disulfiram causes selective hypoxic cancer cell toxicity and radio-chemo-sensitization via redox cycling of copper. *Free radical biology & medicine*. Apr 2020;150:1-11. doi:10.1016/j.freeradbiomed.2020.01.186
22. Capparelli EV, Bricker-Ford R, Rogers MJ, McKerrow JH, Reed SL. Phase I Clinical Trial Results of Auranofin, a Novel Antiparasitic Agent. *Antimicrob Agents Chemother*. Jan 2017;61(1)doi:10.1128/AAC.01947-16

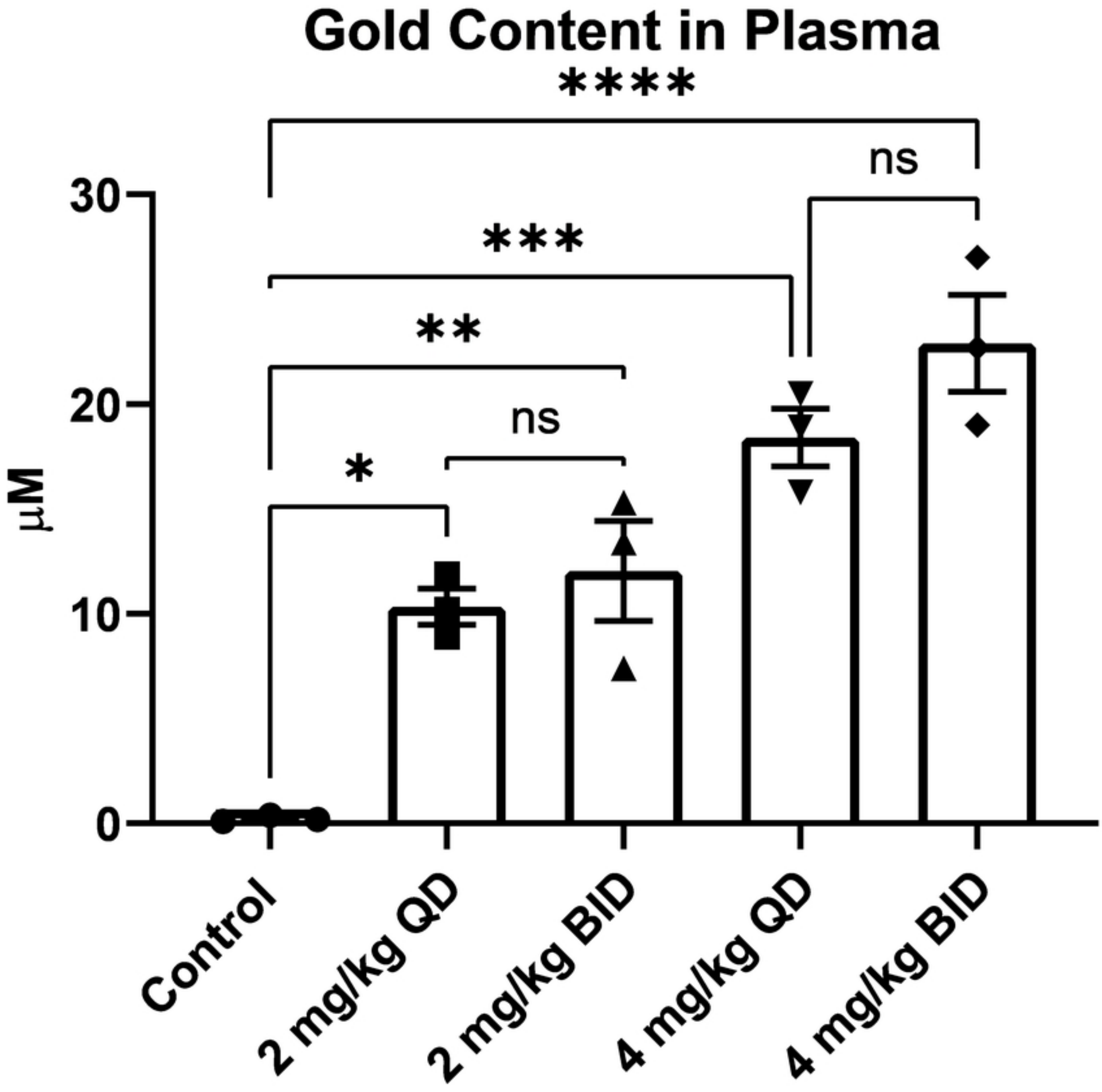
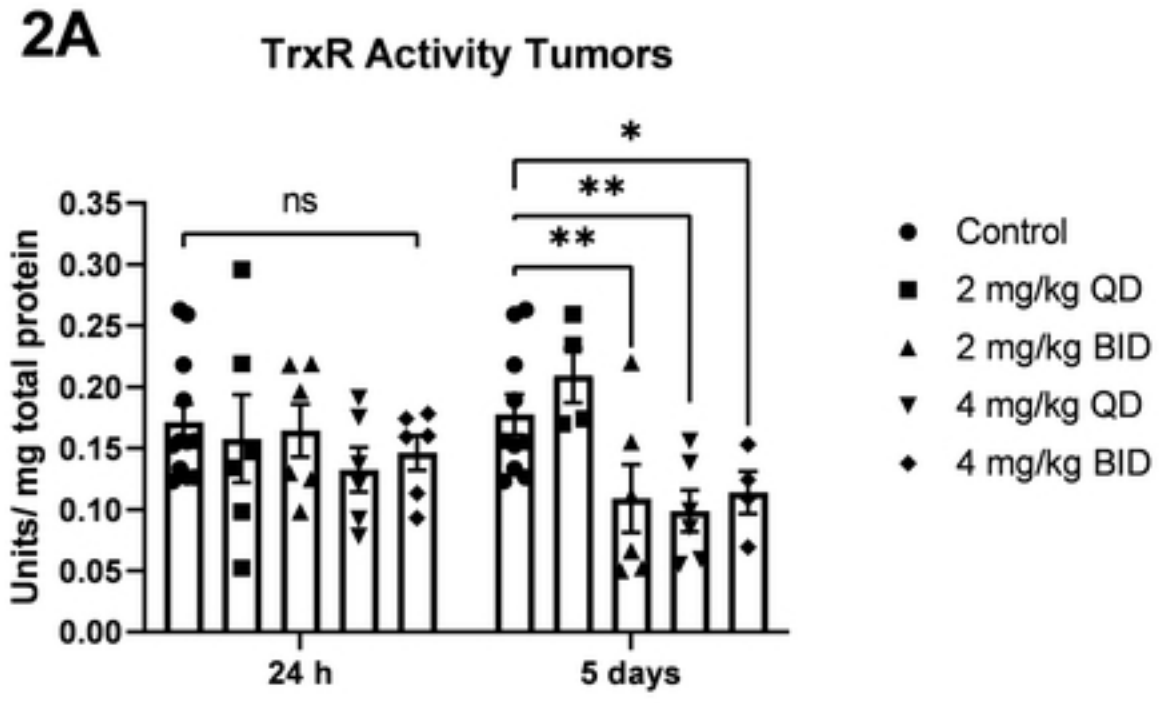

Gold Content in Plasma
****
ns
***
**
ns
*
30
20
10
0
μM
Control
2 mg/kg QD
2 mg/kg BID
4 mg/kg QD
4 mg/kg BID

Figure 1


2A
TrxR Activity Tumors
Units/ mg total protein
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
ns
*
**
**
24 h
5 days
Control
2 mg/kg QD
2 mg/kg BID
4 mg/kg QD
4 mg/kg BID

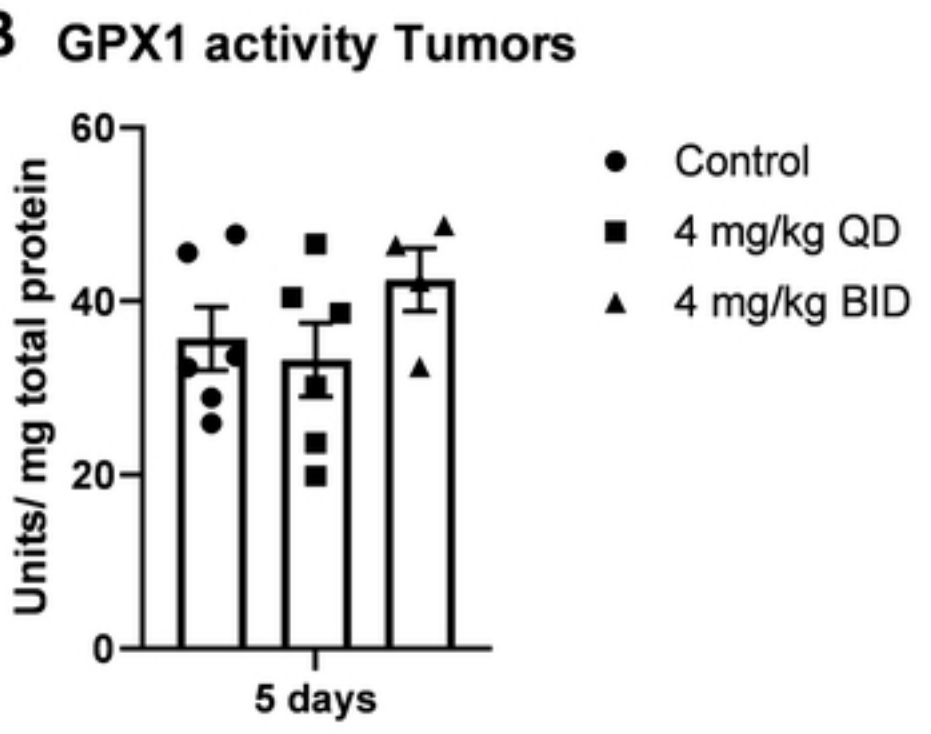

2B
GPX1 activity Tumors
Units/ mg total protein
60
40
20
0
5 days
Control
4 mg/kg QD
4 mg/kg BID

Figure 2

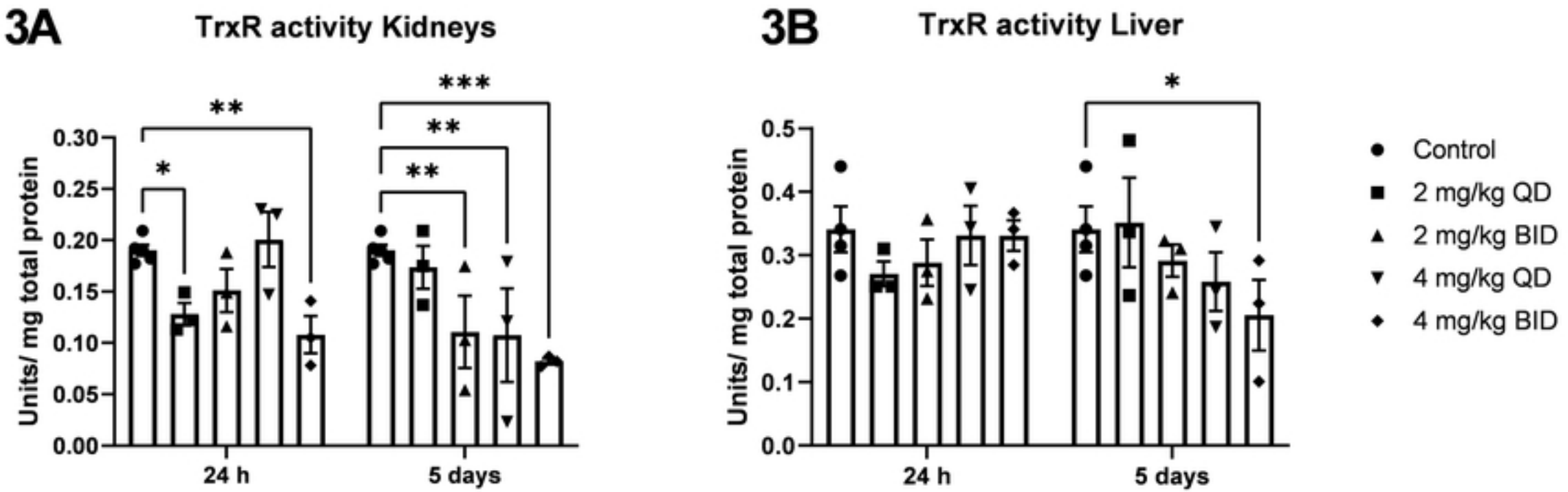

3A
TrxR activity Kidneys
Units/ mg total protein
0.30
0.25
0.20
0.15
0.10
0.05
0.00
*
**
***
**
**
24 h
5 days
3B
TrxR activity Liver
Units/ mg total protein
0.5
0.4
0.3
0.2
0.1
0.0
*
24 h
5 days
Control
2 mg/kg QD
2 mg/kg BID
4 mg/kg QD
4 mg/kg BID

Figure 3

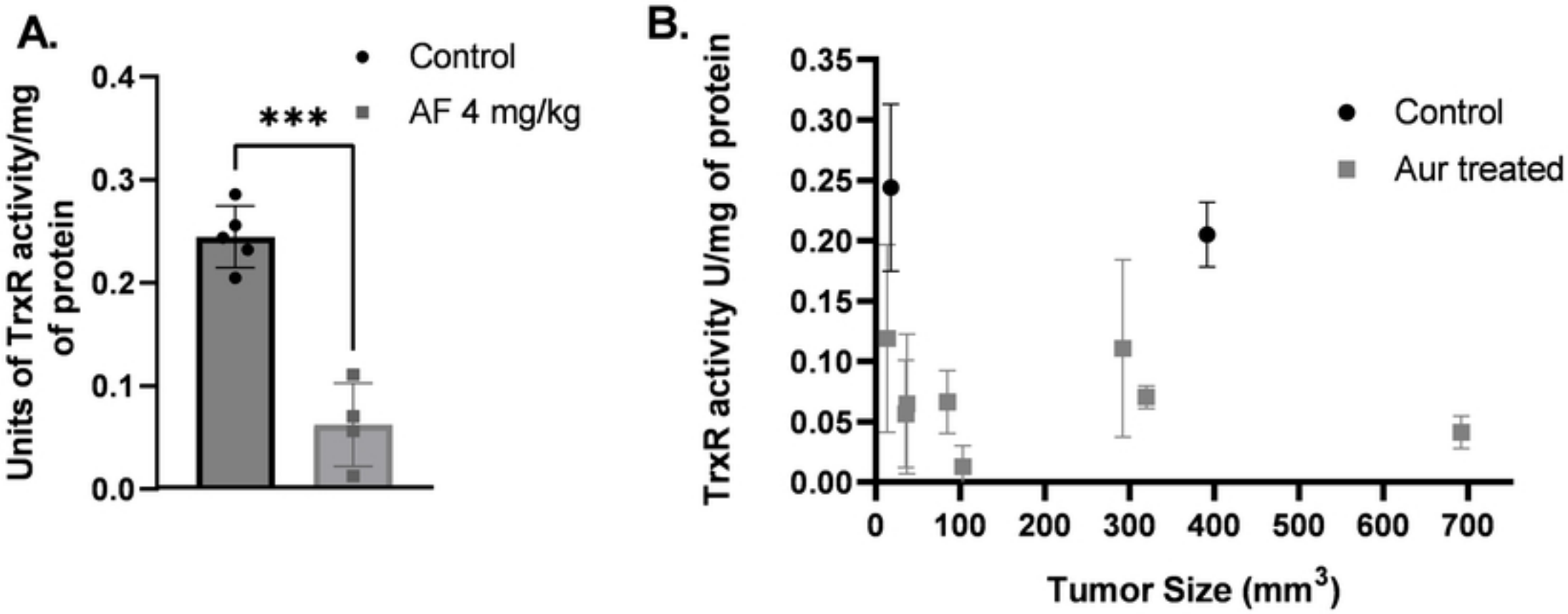

A.
Control
AF 4 mg/kg
***
Units of TrxR activity/mg of protein
0.4
0.3
0.2
0.1
0.0
B.
Control
Aur treated
TrxR activity U/mg of protein
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
100
200
300
400
500
600
700
Tumor Size (mm3)

Figure 4

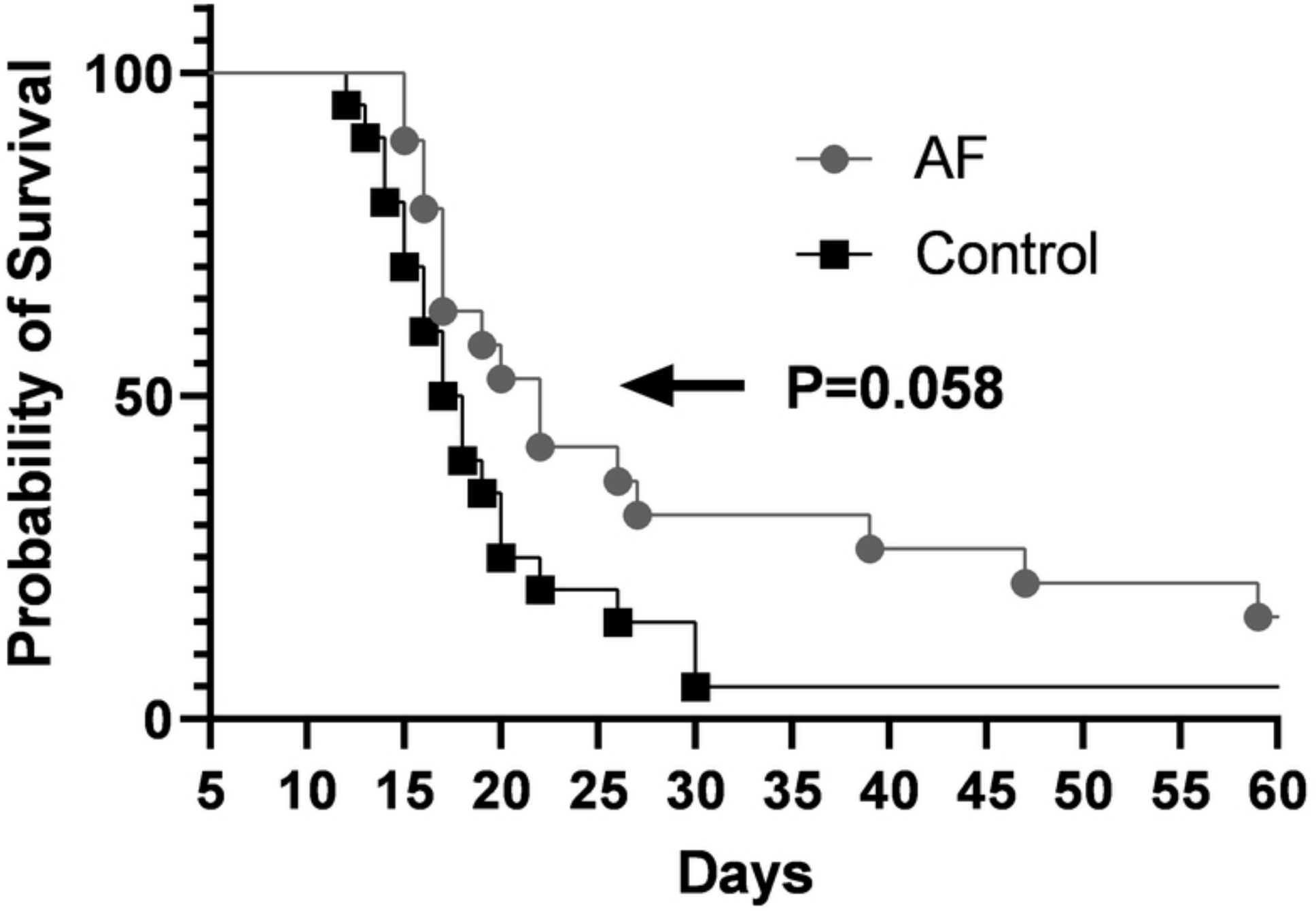

Probability of Survival
100
50
0
5
10
15
20
25
30
35
40
45
50
55
60
Days
AF
Control
P=0.058

Figure 5a

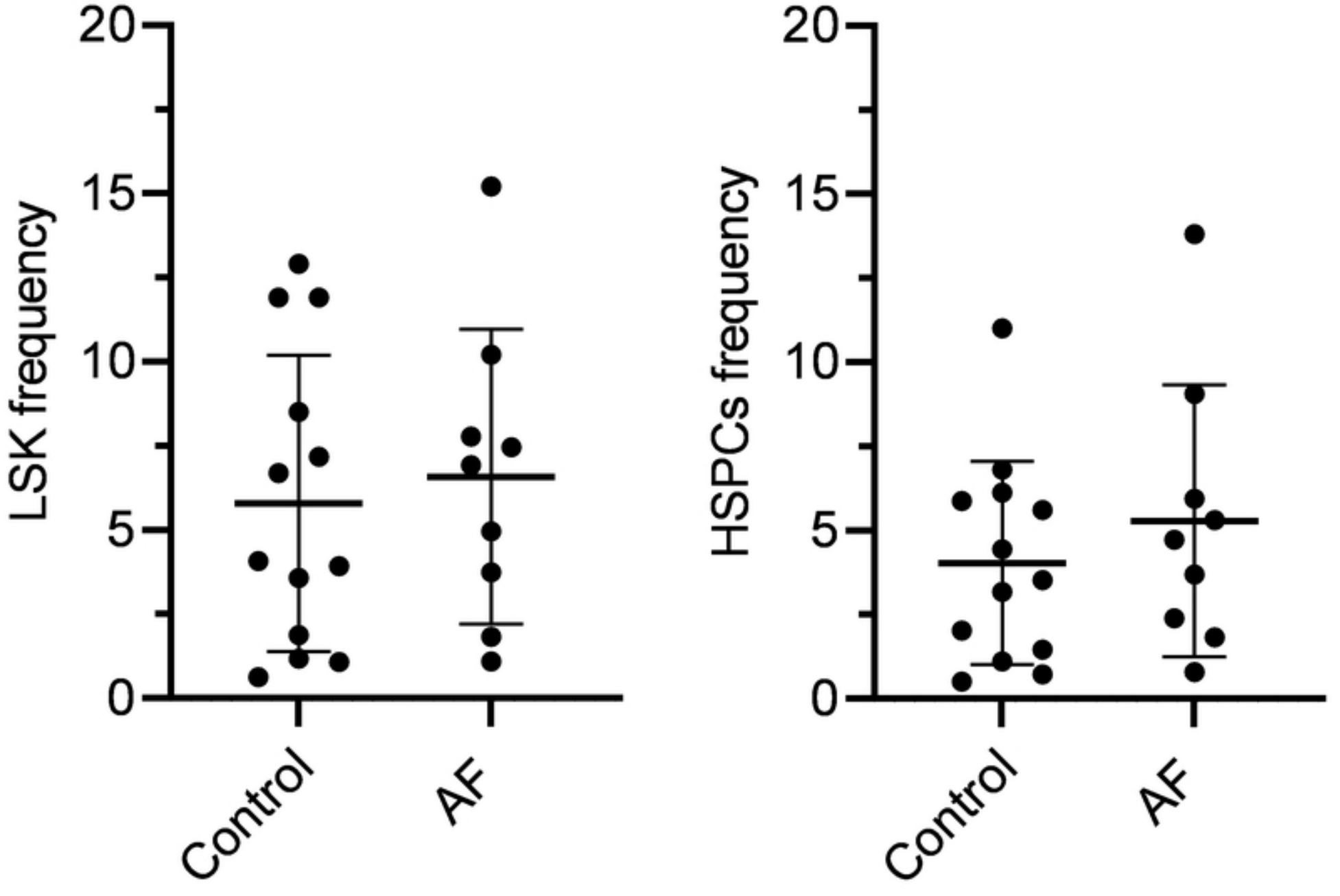

LSK frequency
20
15
10
5
0
Control
AF
HSPCs frequency
20
15
10
5
0
Control
AF

Figure 5b

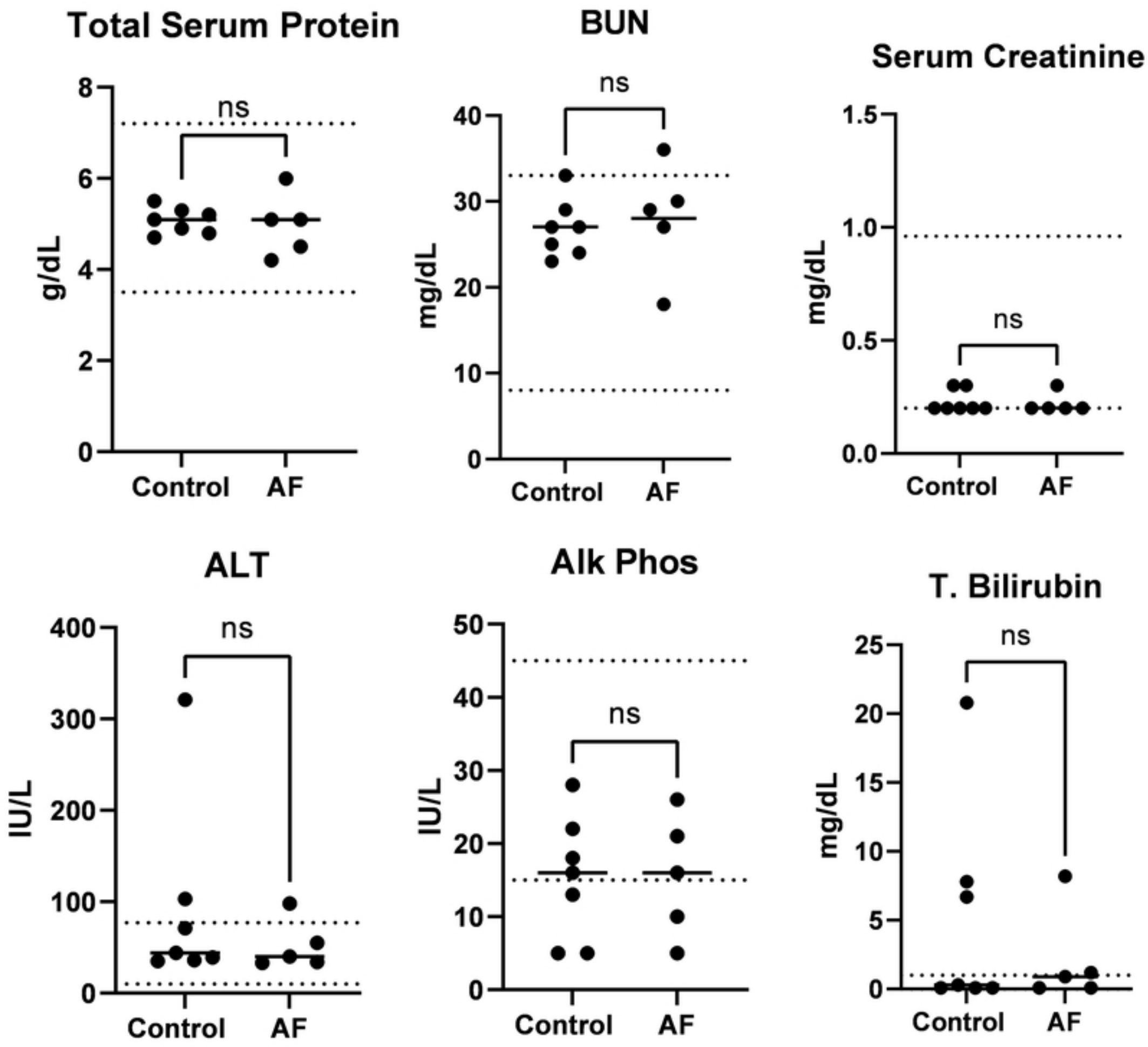

Total Serum Protein
g/dL
8
6
4
2
0
ns
Control
AF
BUN
mg/dL
40
30
20
10
0
ns
Control
AF
Serum Creatinine
mg/dL
1.5
1.0
0.5
0.0
ns
Control
AF
ALT
IU/L
400
300
200
100
0
ns
Control
AF
Alk Phos
IU/L
50
40
30
20
10
0
ns
Control
AF
T. Bilirubin
mg/dL
25
20
15
10
5
0
ns
Control
AF

Figure 5c